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NEUROCHEMICAL ANATOMY OF THE NEUROENDOCRINE HYPOTHALAMUS

Neurochemical anatomy of the hypothalamus

M. PALKOVITS

Summary

Microdissection techniques for isolated removal of the various regions of the hypothalamus as well as the individual hypothalamic nuclei are detailed. Recent development of biochemical microassays have made it possible that the concentrations of neurohormones, neuropeptides, neurotransmitters and their related enzymes could be detected in such a small volume of brain tissue than the hypothalamic nuclei. Data available of the hypothalamic distribution of above substances are summarized. The possible role and origin of intra- and extrahypothalamic neurohormones as well as the existence of the so-called "hypophysiotrophic area" are discussed.

Zusammenfassung

Mikropräparationsmethoden zur Entnahme von winzigen Gewebestücken aus umschriebenen Arealen sowie der individuellen Kerngebiete des Hypothalamus werden im Detail beschrieben. Die moderne Entwicklung der biochemischen Mikromethoden haben es möglich gemacht, die Konzentration von Neurohormonen, Neuropeptiden, Neurotransmittoren und der entsprechenden Enzyme in solch kleinen Gewebsstücken zu erfassen. Die regionale Konzentration der erwähnten Substanzen im Hypothalamus wird kurz zusammengestellt. Herkunftsort und funktionelle Bedeutung der intra- und extrahypothalamischen Neurohormone sowie die Begrenzung der sog. hypophysiotropen Region werden besprochen.

It is known for a long time that the hypothalamus is rich in neurohormones and also in neurotransmitters. The recent development in the sensitivity of neurochemical assays has enabled not only the whole hypothalamus but its discret regions or even the individual hypothalamic nuclei to be measured. This development of biochemical techniques calls for further topographical knowledge of the hypothalamus. Unfortunately, neither a generally acceptable nomenclature nor the exact topographical description of the hypothalamus has been developed yet. Data published by different authors are fairly frequently incomparable since dissections of the hypothalamic regions or nuclei occur more arbitrarily than in accordance with their real distributions. Therefore, the present paper includes a detailed description of the hypothalamic areas with a guide to their isolated removal for neurochemical studies.

Topography and microdissections of the rat hypothalamic areas

Microdissections of the hypothalamic areas can be performed both on fresh and frozen brains either under a dissecting microscope or even by using a simple magnifier. Preparations on fresh brain can be better carried out on the surface of a hard but non-rigid (rubber) back sheet.

Although both topographical and functional observations undoubtedly indicate that the mammillary body and the preoptic region do not belong to the hypothalamus but rather are parts of the limbic system, a number of authors do not separate them from the hypothalamus. Horizontal section at the top of the third ventricle border the hypothalamus dorsally. Perpendicular sections passing through the medial edge of the internal capsule, as well as through the lateral edge of the tuber cinereum separate the hypothalamus from the other parts of the diencephalon (Fig. 1). The rostral (preoptic) border of the hypothalamus is

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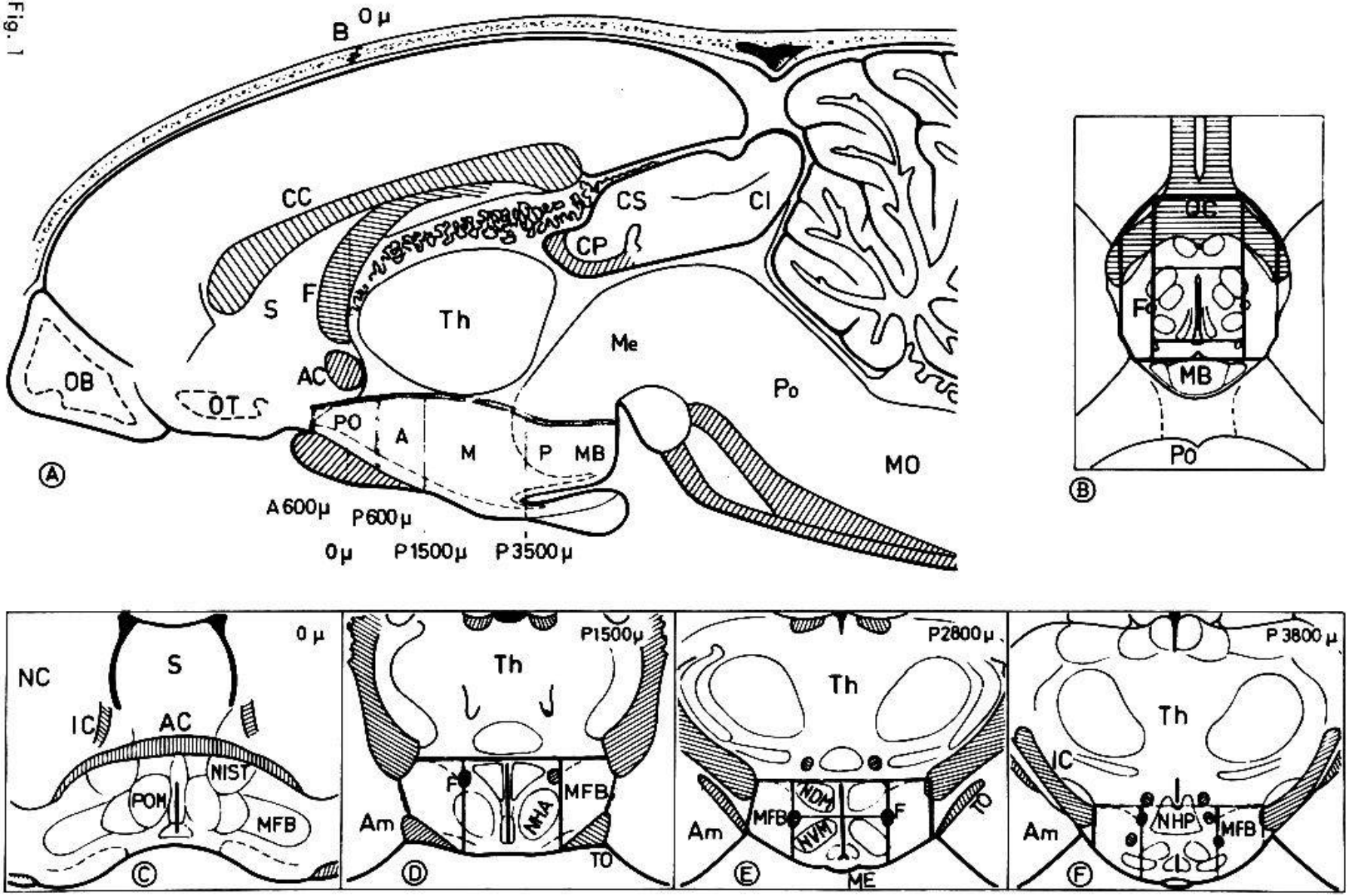
Fig. 1. Schematic drawings of the rat brain and hypothalamus. A - sagittal section of the brain. The hypothalamus and preoptic area are demarcated; B - horizontal view of the diencephalon. The anterior, middle and posterior, as well as the lateral and medial hypothalamic regions are demarcated; C-F - frontal sections of hypothalamus in various distances (rostral=A, caudal=P) from the bregma (B) level (O μ). The medial and lateral hypothalamic areas are demarcated. Abbr.: B - bregma, OB - olfactory bulb, OT - olfactory tubercle, CC - corpus callosum, S - septum, F - fornix, AC - anterior commissure, PO - preoptic region, A - anterior hypothalamus, M - middle part of the hypothalamus, P - posterior hypothalamus, MB - mammillary body, TH - thalamus, Me - mesencephalon, Po - pons, MO - medulla oblongata, CP - posterior commissure, CS - superior collicle, CI - inferior collicle, OC - optic chiasm, NC - caudate nucleus, IC - internal capsule, NIST - nucleus interstitialis striae terminalis, POM - medial preoptic nucleus, MFB - medial forebrain bundle, Am - amygdala, NHA - anterior hypothalamic nucleus, TO - optic tract, ME - median eminence, NVM - ventromedial nucleus, NDM - dorsomedial nucleus, NHP - posterior hypothalamic nucleus.

Fig. 1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600 μ behind the bregma level (Fig. 1A), which is identical with a frontal section bisecting the optic chiasma. The caudal (mammillary) border of the hypothalamus is well observable from the basis of diencephalon (Fig. 1B). This border, however, does not respect the coronal plane since the mammillary nuclei penetrate rostrally into the posterior hypothalamus.

The wet weight of the total hypothalamus of a 200 gr rat is approximately 17,5-18,0 mg.

The hypothalamus can be divided into three parts: anterior, middle and posterior. The anterior hypothalamus starts at the level of 600 μ behind the bregma from rostral, and extends caudally as far as the caudal edge of the optic chiasm (Fig. 1A). This is a well, even macroscopically observable landmark about 1500 μ behind the bregma level in 200 gr rats. The middle hypothalamus starts from here and occupies the major portion of the hypothalamus (Fig. 1A) as far caudal as the separation of the pituitary stalk is (3500 μ behind the bregma). The posterior hypothalamus caudally bordered by the mammillary body is relatively small.

The weights of the hypothalamic regions of 200 gr rats are 6,5 mg (anterior), 7,0 mg (middle) and 4,3 mg (posterior).

All three hypothalamic regions can be further divided by vertical sections passing through the fornix into medial and lateral parts (Figs. 1D-E). This section is about 1,0-1,1 mm lateral to the midline (IIIrd ventricle). The weights of the medial parts of the anterior, middle and posterior hypothalamus are 2,5 mg, 3,6 mg, 1,8 mg and of the lateral parts 4,0 mg, 3,4 mg and 2,5 mg, respectively.

The medial part of the middle hypothalamus can be separated by a horizontal cut bisecting the third ventricle into a basal and a dorsal portion (Fig. 1E). The basal part generally called as medial-basal hypothalamus contains the most important components of the endocrine hypothalamus such as the median eminence, arcuate and ventromedial nuclei. The weight of this portion in 200 gr rats is no more than 1,8 mg.

The isolated removal of the median eminence from fresh brain can be performed by using a fine scissor under a stereo microscope. The stump of the pituitary stalk is fixed and slightly elevated by a forceps and the median eminence is cut out from caudal to rostral direction, bilaterally (G.B. Makara, Budapest, personal communications). An alternative approach which has been used for dissecting the median eminence for biochemical analysis is to remove it by using a fine, sharp forceps. The two shanks of the forceps can be placed straight on both sides of the median eminence and with a light pressure on the basal surface of the brain the median eminence can quasi be plucked out.

The size of the rat median eminence is too small for isolated dissecting of the external and internal layers. This procedure can be effected on the bovine median eminence which can

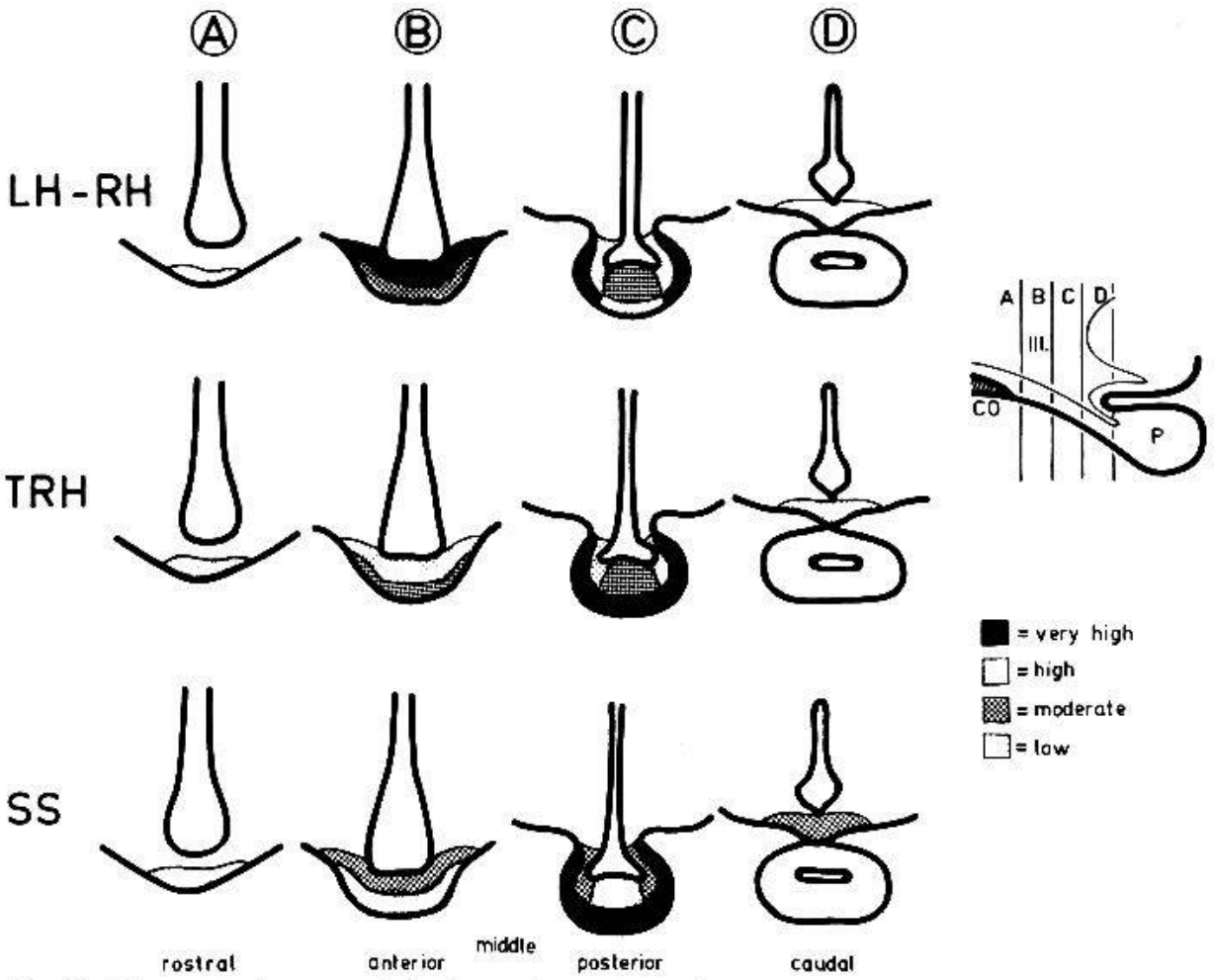

Fig. 2. Schematic drawings of the frontal sections of the bovine median eminence in different rostro-caudal levels (sagittal scheme). The concentrations of luteinizing hormone-releasing hormone (LH-RH), thyrotropin releasing hormone (TRH) and somatostatin (SS) are indicated with different symbols. CO - optic chiasm, P - pituitary gland.

be dissected into eight subdivisions. Serial frontal 300 μ sections are cut from frozen tissue blocks containing the basal hypothalamus and the median eminence. The rostral, anterior, middle and caudal parts of the median eminence can be separated by making knife cuts under a stereo microscope, as described earlier (38). The anterior and middle portions are further subdivided into internal and external layers. The middle portion can be separated once more into medial and lateral parts by making two cuts perpendicular with the floor of the third ventricle (Fig. 2).

The areas and nuclei of the anterior, middle and posterior hypothalamus, as well as their rostro-caudal extensions are summarized in Table 1.

Recent technical developments have enabled discrete hypothalamic nuclei to be removed for performing micro-assays of various substances. The "punch-technique" (53) has proved to be the most suitable one for quick, precise and reproducible dissecting of the hypothalamic

Table I. Rostro-caudal extension of the hypothalamic cell groups

	Nuclei	abbr.	distances from the bregma (0) level in µm rostral	caudal
Anterior hypothalamus	Periventricular nucleus	NPE	560	2290
	Magnocellular periventricular nucleus	NPM	550	910
	Supraoptic nucleus	NSO	380	2800
	Suprachiasmatic nucleus	NSC	600	1340
	Paraventricular nucleus	NPV	1300	2020
	Anterior hypothalamic nucleus	NHA	690	2030
Middle hypothalamus	Retrochiasmatic area	ARC	1380	1990
	Median eminence	ME	1930	3730
	Subfornical nucleus	NSF	1360	2260
	Arcuate nucleus	NA	1700	4520
	Ventromedial nucleus	NVM	1780	3760
	Dorsomedial nucleus	NDM	2530	3540
	Perifornical nucleus	NPF	2450	3300
Posterior hypothalamus	Dorsal premammillary nucleus	NPMD	3650	3960
	Ventral premammillary nucleus	NPMV	3820	4220
	Prelateral mammillary nucleus	NPL	3860	4570
	Posterior hypothalamic nucleus	NPO	3520	4250
	Supramammillary nucleus	NSM	4230	4800

nuclei. The frozen brains are cut in a cryostat of -10°C and the nuclei are removed from the serial frontal sections of the hypothalamus using stainless steel cannula. The inside diameters of the cannula are depending on the size of nuclei to be removed. The sectioning of the brains and the necessary landmarks for the precise dissections of the nuclei are detailed elsewhere (53, 54). The dissecting technique has been adapted for use on fresh sections cut with a modified tissue chopper (79) or with a vibratome (36).

Parallel with the biochemical measurements in the hypothalamic nuclei further studies can be carried out on the separated lobes of the pituitary gland. The isolation of the lobes in fresh pituitary can be performed under a stereo microscope. At first, the anterior lobe is isolated from the intermediate and posterior ones by using a dissecting knife. Thereafter, the posterior lobe is fixed by a fine needle and the intermediate lobe might be detached by a knife meanwhile the tissue is slowly rotated (Fig. 3).

Microassays for biochemical studies on the hypothalamic nuclei

Within the past few years, a number of sensitive and specific micromethods for measuring neurohormones, neurotransmitters and related enzymes has become available. Biochemical techniques which are sensitive enough to determine substances in less than 1 mg brain tissues

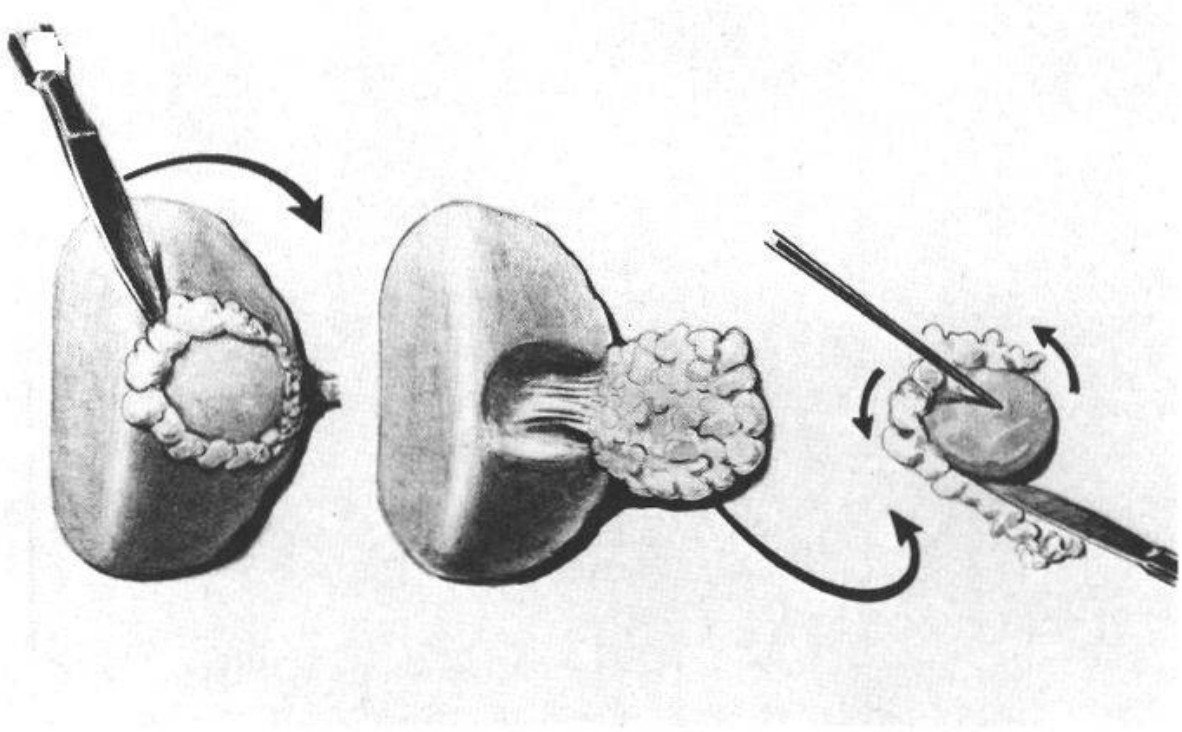

Fig. 3. Separation of the different pituitary lobes in rat.

are suitable for measurements on the hypothalamic nuclei. The recent developed radioenzymatic- and radioimmunoassays, as well as the mass fragmentography have been proved to be the most suitable for that purpose.

The wet weights of the individually removed hypothalamic nuclei are varied between 60 and 1000 µg, in which small amounts of tissues are measurable only with difficulties. Therefore, the determination of the protein contents of the tissue samples (46) seems to be more available and the concentrations or activities of substances measured can be expressed in a ratio of the protein content.

Recently, the distributions of a great number of various neurohormones, neuropeptides, putative neurotransmitter with their synthetizing and katabolic enzymes have been determined in the hypothalamic nuclei. Microassays used for these studies are shown in the Table 2.

Distributions of neurohormones, neuropeptides, neurotransmitters and their related enzymes in the rat hypothalamus

Distributions of these substances in the individual hypothalamic nuclei are summarized in the Table 3. Instead of absolute concentrations of substances measured which have been published elsewhere (16, 55) the Table contains a comparative analysis. The hypothalamic nuclei rank according to the concentrations or activity of substances measured.

Table II. Microassays to measure substances in μg brain tissues

Neurohormones	
LH-RH	: Arimura, Sato, Kumasaka, Wonobec, Debeljuk, Dunn, Schally (1973)
TRH	: Bassiri, Utiger (1972)
Somatostatin	: Arimura, Sato, Coy, Schally (1975)
Oxytocin	: George, Staples, Marks (1976)
Vasopressin	: George, Chapen, Phillips (1972)
Neurotransmitters and neuropeptides	
Noradrenaline and dopamine	: Henry, Starman, Johnson, Williams (1975) Palkovits, Brownstein, Saavedra, Axelrod (1974) Gauchy, Tassin, Glowinski, Cheramy (1976) Zschaeck, Ramirez (1976)
Adrenaline	: Osborne (1973) Van der Gugten, Palkovits, Weinen, Versteeg (1976)
Serotonin	: Cattabeni, Koslow, Costa (1972) Saavedra, Brownstein, Axelrod (1973)
Histamine	: Snyder, Baldessarini, Axelrod (1966) Taylor, Snyder (1972)
Neurotensin	: Brown, Lazarus, Ling, Rivier, Kobayashi, Vale (1977)
Encephalin	: Yang, Hong, Costa (1977) Miller, Chang, Cooper, Cuatrecasas (In press)
Substance P	: Powell, Leeman, Tregear, Niall, Potts (1973)
GABA	: Tappaz, Brownstein, Kopin (1977)
Acetylcholine	: Goldberg, McCaman (1973) McCaman, Stetzler (1977)
Enzymes	
Tyrosin hydr.	: Coyle (1972)
DA-β-hydr.	: Molinoff, Weinshilboum, Axelrod (1971)
Trypt.-hydr.	: Kizer, Zivin, Saavedra, Brownstein (1975)
PNMT	: Axelrod (1972) Saavedra, Palkovits, Brownstein, Axelrod (1974)
Glut. acid. dec.	: Albers, Brady (1959)
Chol. acet. tran.	: Shrier, Shaster (1967) Bull, Oderfeld-Novak (1971)
COMT	: Axelrod, Cohn (1971)
Hist.-N-meth.	: Taylor, Snyder (1972)
MAO	: Wurtman, Axelrod (1963) McCaman, McCaman, Hurt, Smith (1965)

Neurohormones

Distributions and concentrations of five neurohormones in the hypothalamic nuclei of rat have been already determined by radioimmunoassays. (Data about the activities of luteinizing hormone-releasing hormone, somatostatin (73) and of a corticotropin releasing factor measured by bioassays have not been incorporated into the Table 3.)

Table III. Hypothalamic nuclei in order of concentrations or activity of various substances measured by radioenzymatic bioassays

	Neuro-hormones					Neurotransmitters and neuropeptides									Enzymes										
	LH-RH (56)*	TRH (14)	Somatostatin (10)	Oxytocin (27)	Vasopressin (26)	Noradrenaline (59,75)	Dopamine (59, 75)	Adrenaline (74)	Serotonin (66)	Histamine (18)	Neurotensin (40)	Enkephalin (35)	Substance P (13)	GABA (70)	TH (65)	DBH (65)	Tryp.H. (15)	PNMT (67)	GAD (71)	ChAT (12)	COMT (63)	HNM (63)	5HT-MAO (63)	(34)	Tyramine-MAO (34)
Periventricular nucleus	-	3	3	-		3	5	1	12	8			2		3	1	6	8	3	13	2	1	4	6	2
Anterior hypothalamic nucleus	-	14	6	6	-	14	13	-	13	10			4		10	10	3	9	2	9	7	6	6	7	9
Supraoptic nucleus	-	13	14	2	3	6	12	4	14	11			13	4	12	4	12	12	12	6	6	7	8		
Paraventricular nucleus	-	6	10	3	4	2	5	3	11	13			5	1	6	2	11	2	6	8	4	2	12	3	6
Suprachiasmatic nucleus	-	8	7	-	5	9	8	-	3	3			11		8	9	4	11	10	10	11	5	7		
Retrochiasmatic area	-			5	2	4	3		8		4						15	5		12				9	7
Median eminence	1	1	1	1	1	7	1	5	9	1	1		14	7	1	5	8	1	13	1	1	10	5		
Arcuate nucleus	2	5	2	4	6	11	2	6	1	4	3		10	6	5	8	13	4	11	11	8	11	3	5	1
Ventromedial nucleus	3	2	5	-	-	10	10	-	15	9	2	2	9	5	9	6	7	6	5	15	10	12	2	1	5
Dorsomedial nucleus	-	4	8	-	-	1	4	2	10	6		4	1	2	7	3	5	3	1	5	5	4	1	4	8
Perifornical nucleus	-	7	13			8	11	-	2	14			7		4	14	1	7		7	12	9	9		
Ventral premammillary nucleus	-	11	4			13	15	-	7	2			3		13	12	10	14	9	14				2	3
Dorsal premammillary nucleus	-	10	11			15	14	-	6	7			12		14	13	14	15	8	3				11	4
Posterior hypothalamic nucleus	-	9	12	-		12	9	-	5	5		5	8		11	11	9	13	7	2	9	8	11	8	10
Medial forebrain bundle	-	12	9			5	7	-	4	12		3	6	3	2	7	2	9	4	4	3	3	10	10	11

* Ref. in ( )

It is a general rule that the greatest concentrations of all neurohormones measured are found in the median eminence. While the thyrotropin releasing hormone and the somatostatin are present throughout the hypothalamus in relative large amounts, luteinizing hormone-releasing hormone is localized only in the median eminence and the arcuate nucleus. The highest activities of oxytocin and vasopressin are found in the magnocellular hypothalamic nuclei (supraoptic and paraventricular) while in certain other areas they are of intermediate amounts (26, 27). Cell groups of the medial basal hypothalamus are relatively rich in neurohormones, but the periventricular nucleus also ranks in prominent positions (Table 3).

Neuropeptides, neurotransmitters

Pharmacological studies indicate the catecholamines to be involved in central endocrine regulation. Other putative neurtransmitters, notably serotonin, GABA, acetylcholine, substance P and histamine have been found in the rat hypothalamus and median eminence, raising question of relative importance of compounds other than catecholamines in neuroendocrine regulation at the level of the hypothalamus.

Neuropeptides and neurotransmitters measured by radioenzymatic microassays are widely distributed throughout the hypothalamus (Table 3). The concentrations of dopamine, histamine and neurotensin of the median eminence were significantly higher than their concentrations in any other hypothalamic nuclei. With regard to concentration of other neuropeptides or biogenic amines, the median eminence is preceeded by certain hypothalamic nuclei (Table 3). The highest levels of norepinephrine are found in the paraventricular and dorsomedial nuclei. These cell groups are also rich in substance P, adrenaline and GABA. The periventricular nucleus also contains catecholamines and substance P in relative high concentrations. The arcuate nucleus contains not only serotonin and dopamine in a fairly high level but it ranks in the high positions concerning with other neuropeptides and neurotransmitters measured. Concentrations of these substances (except the enkephalin) in the anterior hypothalamic nucleus are quite low (Table 3).

Distribution in the hypothalamus of enzymes associated to neurotransmitters

Enzymes involved in the synthesis and degradation of putative neurotransmitters show relative high activities in the hypothalamic nuclei. Considering the fact that neurotransmitter-producing perikarya are located in small number only in few hypothalamic nuclei but enzym activities are present throughout the hypothalamus speaks in favour of synthesis and degenerations of certain neurotransmitters in the nerve terminals in the hypothalamus.

Several enzyme activities measured (tyrosine hydroxylase, phenylethanol N-methyl transferase, catechol-O-methyl transferase) are of the highest in the median eminence which contains mostly axons and nerve terminals but only an insignificant number of cell bodies. Two enzymes are found in the highest level in the periventricular (dopamin-β-hydrogenase, histamine N-methyl transferase) and dorsomedial (glutamic acid decarboxylase, 5-HT-MAO) nuclei (Table 3). In general, these two cell groups are rich in other enzymes measured too, while low and high activities are varied in other nuclei of the hypothalamus.

General considerations

Neurochemical studies provide important informations concerning distributions of active substances in the endocrine hypothalamus. These data, however, have to be completed with immunocytochemical observations to estimate whether biochemically determined neuropeptides or neurotransmitters are located in perikarya, axons or nerve terminals. Summarizing data available in the recent literature, the following questions will be discussed:

1. Sources of the hypothalamic neuropeptides. How the extrahypothalamic neuropeptides can be transported into the hypothalamus?
2. New explanation of the concept of the "hypophysiotrophic area" (30, 31, 32) with special regard to the existence of extrahypothalamic neurohormones.
3. What is the possible role of neuropeptides measured throughout the endocrine hypothalamus and in the median eminence?

ad 1. Like many other substances, the greatest concentrations of neurohormones in the brain are found in the median eminence. In many instances their contents are significantly with one or two orders of magnitude, higher than that of any other regions of the hypothalamus. The hypothalamus, in general, contains neurohormones also in higher level than the other brain regions, although, in consequence of the large volume of the extrahypothalamic brain, the total amounts of the extrahypothalamic neurohormones exceed the amount found inside the hypothalamus.

Only the minority of neurohormones in the hypothalamus or in the median eminence are of hypothalamic origin. It has been proved by immunocytochemistry that LH-RH, TRH or somatostatin containing (synthetizing) cell bodies are located mostly outside the hypothalamus. Theoretically, these substances can reach the hypothalamus and median eminence by the following ways:

a) Neuronal pathways. It is most likely that extrahypothalamic neurohormones are transported by axons into the hypothalamus. The existence of neurohormone-containing fib-

res has been demonstrated by immunocytochemical techniques. The axonal transportation of neurohormones are also supported by the fact that surgical deafferentation of the medial basal hypothalamus resulted in a decrease of 75-85 % of neurohormones in the region (9, 11, 17, 19, 58, 76).

b) Cerebrospinal fluid (CSF). Recent studies have shown that circumventricular organs, which lay outside of both the blood-brain and brain-liquor barriers, contain neurohormones (37), neurotransmitters and their associated enzymes (64) in fairly high concentrations. These observations give food for a hypothesis, namely that the above substances might get the brain areas through the CSF and vice versa. From the third ventricle neurohormones and neuropeptides might be transported by tanycytes into the portal vessels of the median eminence to reach the pituitary gland. We are still not familiar with the functional significance and capacity of this transport system and the hypothesis calls for further observations.

c) Vascular transportation. Studies on the angioarchitecture of the hypothalamus (2) have been verified that neither the hypothalamus nor the median eminence nor the pituitary gland have direct vascular connections with any other regions of the central nervous system. In opposite, substances from the pituitary gland might be transported back to the arcuate nucleus. The anatomical base of this possible backflow is the special vascular structure of the median eminence (2). Under the floor of the third ventricle, a fine subependymal arterial plexus emerges from the vascular loops of the pituitary arteries in the median eminence and gives terminal branches to terminate in the capillary plexus of the arcuate nucleus. The subependymal plexus is connected with the pituitary vessels on the dorsal surface of the pituitary stalk. Through this, vascular pathway substances from the pituitary gland can reach the median eminence as well as the arcuate nucleus. Recently, it has been demonstrated that peptides (ACTH analogs) injected directly into the pituitary gland have been transported back to the hypothalamus (49). This pathway, however, might be important in the pituitary-hypothalamic feedback mechanisms rather than in neuropeptide transportations since no significant changes in the levels of certain peptides compared to the control values were found in hypophysectomized animals (43, 41).

ad 2. The existence of extrahypothalamic neurohormones contradicts the exclusive role of the "hypophysiotrophic area" in the neurohormonal regulation of the pituitary gland. It has been verified recently that 1.) the neurohormones-containing perikarya are located mostly or totally out of the hypophysiotrophic area; 2.) the major percentage (75-85 %) of neurohormones in the medial basal hypothalamus disappears following a total surgical

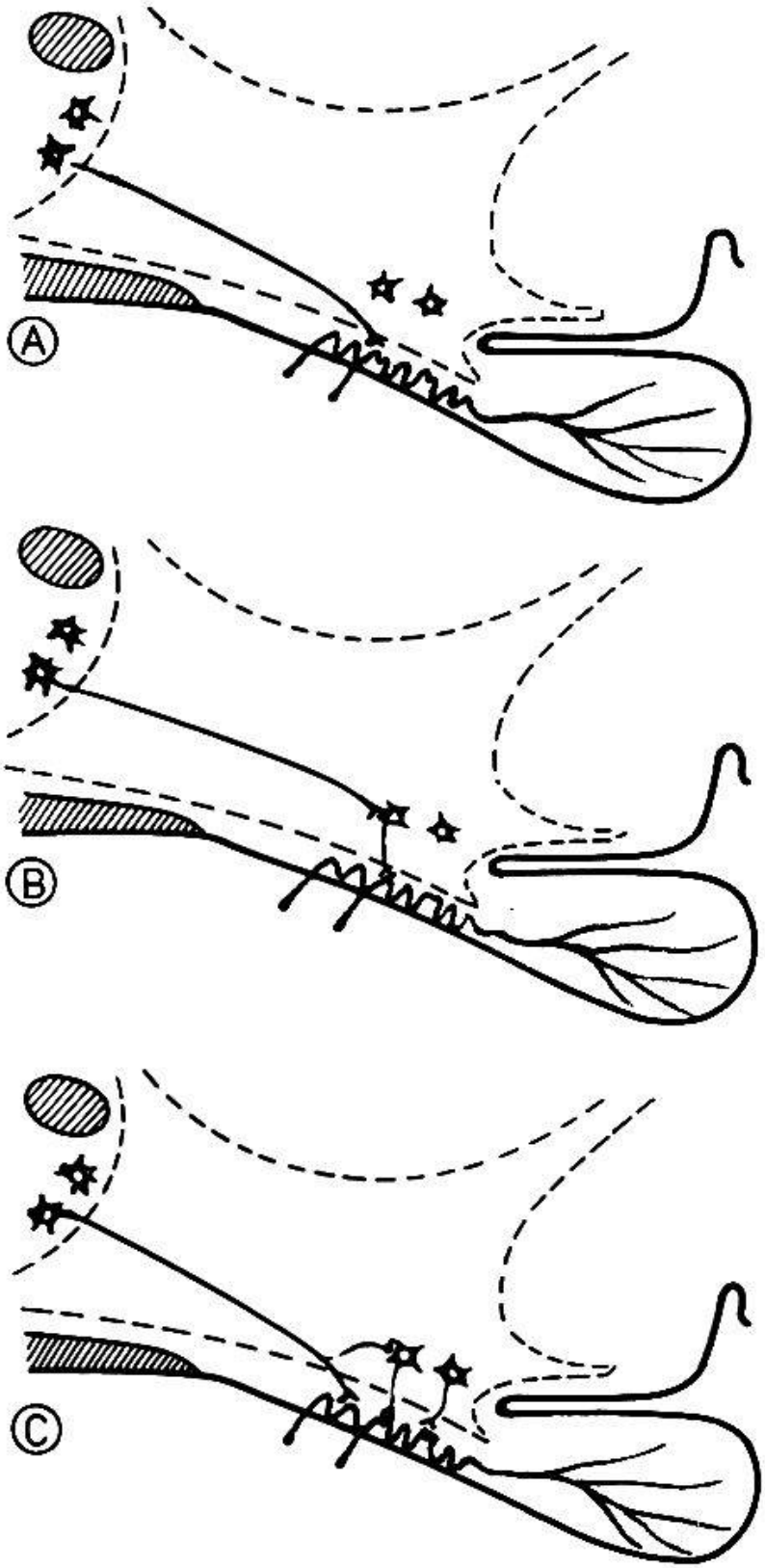

Fig. 4. Theoretical variations of the termination of the extrahypothalamic "neurohormone"-containing neurons. A - Nerve terminals on the capillary loops of the median eminence (neurohormonal effect). B - Nerve terminals on the hypothalamic neurons (neurotransmitter or modulator effects). C - Axon collaterals and nerve terminals both on the capillary loops of the median eminence and hypothalamic neurons.

deafferentation of this region, and 3.) extrahypothalamic axons and nerve terminals have been demonstrated in the median eminence by electron microscopic and autoradiographic studies.

In spite of the above observations, the hypophysiotrophic area is a fact but with an other meaning as has been used. Pituitary grafts could survive and show functional activity after having transplanted into the medial basal hypothalamus but not into any other hypothalamic regions. This observation, however, does not necessarily mean the releasing or inhibiting hormones to be produced in the hypophysiotrophic area, but shows its trophic effect on the pituitary tissue. One of the possible explanations is that the medial-basal hypothalamus, but exclusively only this hypothalamic region, is supplied by the same vessels which supply the pituitary gland, in situ. It is unknown whether the hypophysiotrophic effect of this area is related to the presence of certain substances in the arcuate blood or simply, that the special structure of these vessels can ensure a better blood supply for the grafts than elsewhere.

ad 3. Since there is only an insignificant number of neurons, the neurohormones and neurotransmitters measured in the median eminence are supposed to be located in axons and nerve terminals. Similarly, neurohormones are found in various concentrations throughout the hypothalamus, but only few or no neurohormone-synthetizing cells have been found in the hypothalamic nuclei. This fact means that "neurohormones" should be also located in axon terminals raising the question of their local functional role. At the nerve terminal levels neurohormones do not act like hormones rather as neurotransmitters effecting on an other neuron which penomena can be demonstrated with electrophysiological techniques (61). Independently of the terms of transmitters or modulators (29), their presence in axon terminals suggest that depending on the site of action, substances in the central nervous system might have both hormonal and transmitter characters. Further questions are to be answered whether they are neurons which produce only "hormonal" or only "transmitter" neurohormones or both of them. In the first case, axons terminate on the capillary loops of the median eminence (Fig. 4A), in the second one on the surface of a hypothalamic neuron (Fig. 4B). We still cannot provide direct anatomical evidences for the first or second variation, but studies on the extrahypothalamic neurons using Golgi-impregnation technique give an impression that the high number of axon collaterals and well-developed axon arborizations pattern in the hypothalamus might indicate the parallel existence of both versions (Fig. 4C). In that case, the terms of neurohormones or neurotransmitters do not distinguish different substances but different effects of same substances depending on the site of their actions.

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